BIOSYNTHESIS OF SERINE IN *ESCHERICHIA COLI* AND *SALMONELLA TYPHIMURIUM*

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ABSTRACT

UMBARGER, H. E. (Long Island Biological Association, Cold Spring Harbor, N.Y.), MERLE A. UMBARGER, AND PATRICK M. L. SIU. Biosynthesis of serine in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **85**:1431–1439. 1963.—Evidence for the operation in extracts of *Escherichia coli* of a pathway from glucose to serine involving 3-phosphoglycerate, phosphohydroxypyruvate, and phosphoserine as intermediates was obtained by the technique of isotopic competition. The steps of the pathway were demonstrated in extracts of *E. coli* and *Salmonella typhimurium.* The first reaction was studied in the reverse of the biosynthetic direction by observing the disappearance of reduced nicotinamide adenine dinucleotide in the presence of phosphohydroxypyruvate. The enzyme catalyzing this reaction was missing in two *E. coli* mutants that required serine or glycine for growth and in a representative of one of two genetically distinct classes of *S. typhimurium* serine-glycine auxotrophs. The second reaction, the amination of phosphohydroxypyruvate, was also studied in the reverse of the biosynthetic direction using α-ketoglutarate as the amino acceptor in a transamination reaction with phosphoserine. The final step, the cleavage of phosphoserine, could not be catalyzed by extracts prepared from cells of *S. typhimurium* serine-glycine auxotrophs of the second genetic class. It has been concluded that these three reactions provide the only significant pathway to serine in these organisms.

In animal and plant tissues, there is strong evidence that the biosynthesis of serine proceeds via 3-phosphoglycerate, phosphohydroxypyruvate, and phosphoserine (Ichihara and Greenberg, 1957; Hanford and Davies, 1958). There is also evidence for the existence of a nonphosphorylated pathway (via glycerate and hydroxypyruvate) in animal tissues (Willis and Sallach, 1962). It is not known at present whether both pathways exist in the same tissue or whether different tissues vary in regard to the predominance of one pathway over the other.

In several species of bacteria, the possibility of more than one pathway is clearly eliminated by the frequent occurrence of mutations resulting in a nutritional deficiency that can be satisfied by either serine or glycine. There is, therefore, only one significant pathway leading from glucose to serine and glycine. Although neither mutant methodology nor isotope studies have provided proof that serine is formed from glycine or vice versa, the experiments with *Escherichia coli* of Roberts and his colleagues (1955) appear to provide the best evidence that serine precedes glycine. However, evidence for or against the 3-phosphoglycerate pathway was not obtained in their isotope-competition studies owing to the inability of the intermediates to enter the cell. Evidence for the occurrence in *E. coli* of the conversion of phosphohydroxypyruvate to phosphoserine and the cleavage of the latter to serine, however, has been obtained by Smith et al. (1956).

In this paper, a procedure is described in which the general technique of isotope competition in growing cells, as developed by Roberts and his colleagues, has been extended to a system utilizing disrupted cells. Direct evidence in support of the 3-phosphoglycerate pathway in *E. coli* was thus obtained. In addition, the examination of extracts derived from wild-type and serine-glycine auxotrophic strains of *E. coli* and *Salmonella typhimurium* revealed the obligatory nature of the pathway.

MATERIALS AND METHODS

The organisms employed in these experiments were four strains of *E. coli* and three of *S. typhimurium.* The *E. coli* strains were the K-12

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wild-type organism and three auxotrophs derived from it: strain JHM-544, an isoleucine auxotroph which lacked threonine deaminase (Umbarger and Brown, 1957), and strains 119 HM-1 and 129 HM-1, both of which were serine-glycine auxotrophs. The *S. typhimurium* strains were the wild-type strain LT-2 and two serine-glycine auxotrophs derived from it: strains *ser A* 4 and *ser B* 10, kindly provided by M. Demerec.

The medium employed was that of Davis and Mingioli (1950) modified by the omission of citrate and by increasing the glucose concentration to 0.5%. For the growth of the serine-glycine auxotrophs, the medium was supplemented with 70 mg of L-serine per liter. Phosphate was determined by the method of Fiske and SubbaRow (1925).

For the extracts employed in the isotope-competition studies, *E. coli* strain JHM-544 was grown in a medium supplemented with excess L-isoleucine (100 mg per liter). The cells were harvested, while in the logarithmic phase of growth, by centrifugation and washed twice in 0.05 M potassium phosphate buffer (pH 7.0). The washed cells were suspended in eight times their wet weight of the same buffer and disrupted by means of a Mullard 20-kc ultrasonic oscillator. The extract thus obtained was clarified by centrifugation at 25,000 × *g* for 20 min. Extracts used for the demonstration of the enzymatic steps were prepared in a similar way, except that 0.01 M tris(hydroxymethyl)aminomethane (tris)-HCl buffer was employed both for washing and for disrupting the cells. Although the wild-type parental strains were harvested while in the logarithmic phase, the serine-glycine auxotrophs were harvested after growth had ceased owing to the depletion of the L-serine supplement (70 mg per liter). This procedure was arbitrarily employed to allow for maximal formation of the enzymes if they were subject to control by end product repression.

In the isotope-competition studies using extracts, the amino acids formed during the reaction were isolated by adsorption on and elution from Dowex-50. They were separated by two-dimensional descending paper chromatography. The first solvent was freshly prepared *n*-butanol–formic acid (85%, w/v)–water (90:10:24). The papers were equilibrated first for 3 hr in an atmosphere saturated with a 15% solution of the same solvent in water. The second solvent was phenol saturated with 6.3% potassium dihydrogen phosphate and 3.7% trisodium citrate·$2H_2O$ (Berry et al., 1951). The solvent was added after the papers had been equilibrated for 3 hr in an atmosphere saturated with 3 N ammonia.

The positions of the radioactive amino acids were located by radioautography, employing the techniques recommended by Roberts et al. (1955). Counts in the amino acids were made by placing the probe of a Tracerlab TGC-14 thin window Geiger-Mueller tube directly over the area on the chromatogram to be counted, using as a shield the corresponding X-ray film from which the appropriate darkened area had been excised. Background areas of the same size were counted on either side of the radioactive spot, and the values were averaged to supply a correction for the count made over the radioactive spot.

Uniformly labeled glucose-C^{14} was obtained from New England Nuclear Corp., Boston, Mass. The α-ketoglutarate-C^{14} was prepared from DL-glutamate-1-C^{14} (also obtained from New England Nuclear Corp.) by transamination in the presence of α-ketoisovalerate and an *S. typhimurium* extract that exhibited a derepressed level of transaminase B (Rudman and Meister, 1953). The reaction mixture was extracted with ether and most of the original α-ketoisovalerate was removed by evaporation in vacuo. Phosphohydroxypyruvate, pyridoxal phosphate, and the amino acids employed were obtained from Calbiochem. All other fine chemicals and cofactors were obtained from Sigma Chemical Co.

RESULTS

Serine biosynthesis from glucose by an E. coli extract. In view of the impermeability of the intermediates in the pathway leading from 3-phosphoglycerate to serine, the role of this pathway could not be established by the technique of isotope competition. Therefore, an attempt was made to demonstrate the over-all conversion of glucose to serine in a bacterial extract. It was assumed *a priori* that the demonstration might be difficult for two reasons. One was the fact that *E. coli* exhibits appreciable levels of threonine (and serine) deaminase activity when grown in a mineral salts glucose medium (Umbarger and Brown, 1957). The other was that as soon as a very small amount of serine was formed further synthesis would be quenched by end product inhibition. This latter possibility seemed quite likely in view of the fact that Roberts et al. (1955) had shown that serine is one amino acid that is not overproduced and, when present exogenously, is preferentially utilized. This difficulty was overcome by using small amounts of glucose with a high specific activity. In an effort to overcome the possibility of an interference by serine deamination, a strain of *E. coli* (JHM-544) was employed which has almost no serine deaminase activity (Umbarger and Brown, 1957). In a subsequent experiment employing an extract of a wild-type strain of *E. coli* (K-12), it was observed that this precaution was unfounded. Serine formation with this extract was comparable with that with the extracts prepared from strain JHM-544.

In a preliminary experiment, an extract prepared from this strain was incubated for 30 min in a 1.0-ml system containing 2 μmoles of glucose-U-C^{14} (specific activity, 6.25 μc per μmole), 20 μmoles of L-glutamate as an amino donor, and other supplements. At the end of the incubation period, 0.75 ml of the trichloroacetic acid-soluble fraction was adsorbed on small columns containing about 2 ml of Dowex-50-H^+ (200 mesh, 8% cross linkage). The columns were washed with 10 volumes of water and then eluted with 4 volumes of 6 N ammonia. The eluates were evaporated to dryness in a Buchler evapo-mix and taken up in water; one-half of each sample was applied to the paper and chromatographed, as described in Materials and Methods.

In this experiment, the radioautograph prepared from the chromatogram indicated radioactive areas corresponding in position to alanine, valine (except as noted below), aspartate, serine, and glucosamine. Other radioactive areas were much weaker. As may be seen in Table 1, the radioactivity in serine was strikingly less than in alanine. Furthermore, when the label in serine was compared with that in alanine, there did not seem to be any correlation. However, if the radioactivity in valine was added to that in alanine, a strong correlation occurred. The data revealed that the omission of either nicotinamide adenine dinucleotide phosphate ($NADP^+$) or diphosphothiamine (DPT) did not affect the formation of serine. Nicotinamide adenine dinucleotide (NAD^+), however, appeared to be necessary since its omission resulted in a 55% suppression of incorporation. The formation of valine was virtually blocked by omitting DPT. This observation is of interest in view of the fact that the initial step in valine biosynthesis is catalyzed by an enzyme with a very low affinity for DPT (Umbarger and Brown, 1958). In the second part of Table 1, the relative labeling of serine is compared with that in alanine plus valine and with that in serine in the complete system.

TABLE 1. *Conversion of glucose-U-C^{14} to serine by an Escherichia coli extract**

System	Counts per min			Per cent incorporation into serine	
	Alanine	Valine	Serine	Relative to alanine + valine	Relative to complete system
Complete	28,490	10,431	683	1.75	100
$NADP^+$ omitted	37,080	10,544	809	1.70	97
$NADP^+$ omitted	41,069	560	697	1.68	96
NAD^+	22,933	7,668	240	0.78	45

* The complete system contained in 1.0 ml: 100 μmoles of potassium phosphate (pH 7.5); 20 μmoles of magnesium chloride; 5 μmoles of sodium adenosine triphosphate; 80 μg of diphosphothiamine; 0.1 μmole of NAD^+; 0.1 μmole of $NADP^+$; 20 μmoles of sodium glutamate; 20 μg of pyridoxal phosphate; 2 μmoles of glucose-U-C^{14} containing 12.5 μc; 0.4 ml of crude extract prepared from *E. coli* strain JHM-544. After 30 min at 37 C, the reaction was stopped by adding 0.1 ml of 50% trichloroacetic acid. For other conditions, see text.

Competition between glucose and serine precursors. With the demonstration that serine could be formed from a general carbon source in a system without the permeation barrier of whole cells, it became possible to test the intermediates in the 3-phosphoglycerate pathway as competitors of the incorporation of glucose carbons into serine. Accordingly, extracts were again prepared and incubated in the system from which DPT and $NADP^+$ had been omitted. In experimental tubes, various competitors were added. The data, expressed as the activity relative to that in the control, are given in Table 2. In each case, the data were normalized with respect to the incorporation into alanine alone since valine was virtually absent. As the table shows, only phosphoserine and phosphohydroxypyruvate exhibited an intense quenching of radioactivity in serine. In contrast, glycerate, a proposed

TABLE 2. *Effect of several compounds on the incorporation of glucose carbons into serine**

Competitor added	Relative activity in serine
None	100
Phosphohydroxypyruvate acid	9
Phosphoserine	6
Glyceric acid	94
Fructose-1,6-diphosphate	73
Glucose-6-phosphate	61
3-Phosphoglyceraldehyde	93

* The complete system was the same as that in Table 1 except that $NADP^+$ and diphosphothiamine were omitted. The competitors were added as neutral sodium salts in 1-μmole amounts.

intermediate in the nonphosphorylated pathway leading to serine, had no effect. Intermediates in the glycolytic pathway such as glucose-6-phosphate and fructose-1,6-diphosphate had a much smaller effect on the relative incorporation into serine. On the other hand, glyceraldehyde-3-phosphate, a compound which would be expected to follow the branch point in the pathway to serine, had no significant effect.

The data clearly indicated that phosphohydroxypyruvate and phosphoserine are likely precursors of serine. An additional observation provided even more convincing evidence. When the chromatograms were treated with ninhydrin in the usual manner, the amount of serine formed, although sufficient to provide a darkening on the radioautograms, was not enough to yield a ninhydrin spot. Presumably, the small amount of serine formed was enough to quench further metabolite flow along the pathway to serine through end product inhibition of some step in the pathway. When phosphohydroxypyruvate and phosphoserine had been present during the incubation, however, a vivid ninhydrin spot in the region corresponding to serine was formed, even though the darkening on the radioautogram was detected only with difficulty. Clearly, these competitors have bypassed the effect of serine on its own biosynthetic pathway and must therefore lie after the step sensitive to the end product. In contrast, the two glycolytic hexose phosphates tested, although competing to some extent with glucose carbon, did not effect this kind of increase in the serine formed. These competitors, then, preceded the reaction sensitive to the end product. The isotope-competition studies therefore led to the prediction that the conversion of 3-phosphoglycerate to phosphohydroxypyruvate was itself the step sensitive to the end product and that the enzyme catalyzing this reaction would be inhibited by serine.

Conversion of 3-phosphoglycerate to phosphohydroxypyruvate. When extracts of *E. coli* and of *S. typhimurium* were first examined for the reactions of the 3-phosphoglycerate pathway, all the attempts to demonstrate a reaction converting 3-phosphoglycerate to phosphohydroxypyruvate in the presence of $NADP^+$, NAD^+, and even several NAD^+ analogues were unsuccessful. Neither was it possible to obtain more than a slight indication of a reaction when 2,6-dichloroindophenol was used as a hydrogen acceptor. These negative results led to the isotope-competition experiments described above. However, because those results pointed so clearly to the 3-phosphoglycerate pathway, the effort to demonstrate the reaction was renewed. For this purpose, *S. typhimurium* extracts were prepared by use of serine auxotrophs for which considerable evidence indicated that this reaction was probably present. It was hoped, thereby, to be able to obtain extracts with increased amounts of enzyme by growing the cells with limiting amounts of L-serine. Indeed, with such extracts, it was possible to demonstrate the reaction but only in the reverse of the biosynthetic direction by observing the disappearance of phosphohydroxypyruvate in the presence of excess reduced NAD^+ (NADH; Umbarger and Umbarger, 1962).

A more convenient method of following the reaction has more recently been employed in which NADH disappearance is followed spectrophotometrically. An important feature in the successful application of this method was the use of sufficiently high concentrations of NADH (e.g., 6×10^{-2} M), resulting in optical densities in excess of 1.3 when using cuvettes with a 1-cm light path. Figure 1 is the reproduction of the optical density changes recorded on a Zeiss monochrometer equipped with a Gilford optical density converter and recorder. Curves A and B show the changes in optical density in the presence and absence of phosphohydroxypyruvate, respectively, observed with a crude extract prepared from *S. typhimurium* strain *ser B* 10. Curves C and D show the corresponding changes with an extract in which much of the NADH oxidase activity in the crude extract

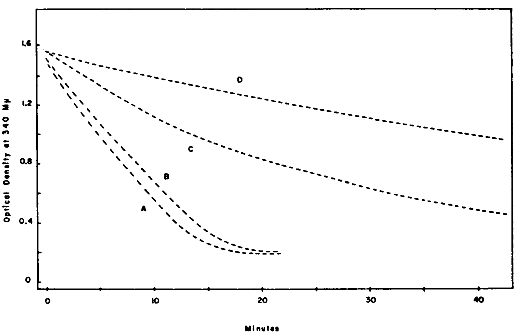

FIG. 1. *Disappearance of reduced nicotinamide adenine dinucleotide (NADH) in the presence of phosphohydroxypyruvate. Each cuvette contained 600 μmoles of tris-HCl buffer (pH 8.7), 4 μmoles of magnesium chloride, and 0.8 μmoles of NADH. In addition, cuvettes A and B contained 0.1 ml of crude extract, with 1.02 mg of protein prepared from Salmonella typhimurium ser B 10. Cuvettes C and D contained 0.1 ml of supernatant with 0.76 mg of protein obtained by centrifuging the crude extract at 100,000 × g for 1 hr. Cuvettes A and C contained 0.6 μmoles of sodium phosphohydroxypyruvate. The volume was 3.0 ml and the temperature was 25 C.*

had been removed by centrifugation for 1 hr at 100,000 × *g*. The reason that the differences between the rates of NADH oxidation in the presence and in the absence of phosphohydroxypyruvate by the extract centrifuged at 25,000 × *g* was smaller than the difference exhibited by the extract centrifuged at 100,000 × *g* is not clear. This observation was a consistent one, however. For this reason, the centrifugation step was routinely employed.

Interconversion of phosphohydroxypyruvate and phosphoserine. In preliminary experiments, the transamination reaction between phosphohydroxypyruvate and glutamate and the reverse reaction between phosphoserine and α-ketoglutarate in reaction mixtures were demonstrated by use of paper chromatographic techniques. One method for the quantitative determination of the extent of transamination between phosphoserine and α-ketoglutarate involves the determination of the glutamate formed by means of glutamic dehydrogenase (Umbarger and Umbarger, 1962). Another method has more recently been developed which may be of general applicability. This method involves the isolation of the amino acid fraction after a transamination reaction between phosphoserine and α-ketoglutarate-1-C^{14}. Since glutamate would be the only labeled compound in this fraction and would have the same specific activity as the added α-ketoglutarate, the radioactivity of this fraction would provide a direct measurement of the extent of transamination.

For this measurement, 0.75-ml samples of the 1.1-ml deproteinized reaction mixtures were placed on small Dowex-50-H^+ columns, and the amino acid fraction was eluted with ammonia as described earlier. After the eluates were evaporated to dryness, the residues were dissolved in 0.2 ml of water, and 0.1 ml was placed on filter paper squares and counted in a Tracerlab liquid scintillation counter.

Figures 2 and 3 show the effect of time and of extract concentration, respectively, on the transamination reaction. In subsequent experiments, specific activities were determined by incubating up to 1.5 mg of protein with the complete system for 20 min.

Conversion of phosphoserine to serine. The activity of this enzyme was measured by de-

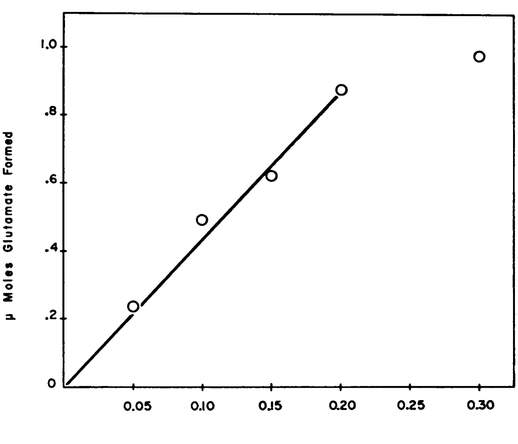

FIG. 2. *Effect of extract concentrations on the extent of transamination between α-ketoglutarate and phosphoserine. The experimental tube contained, in a total volume of 1.0 ml: 100 μmoles of potassium phosphate (pH 8.0), 20 μg of pyridoxal phosphate, 40 μmoles of* DL*-phosphoserine, 20 μmoles of sodium α-ketoglutarate-1-C^{14} with 2.63 × 10^5 disintegrations per min per μmole and, as indicated, an extract prepared from Salmonella typhimurium strain ser B 10 and containing 9.5 mg of protein per ml. After 30 min at 37 C, the reaction was stopped by adding 0.1 ml of 50% trichloroacetic acid. Phosphoserine was omitted from the control tubes. For analytical method, see text.*

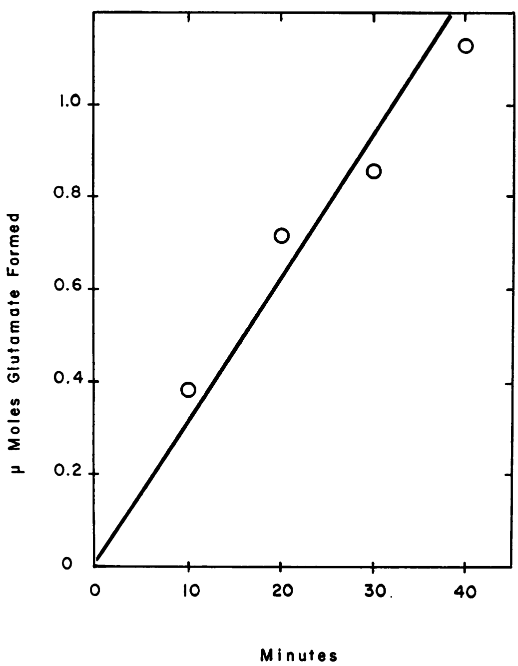

FIG. 3. *Effect of time on the extent of transamination between α-ketoglutarate and phosphoserine. Except for extract, which was 0.6 ml, the experimental tube contained four times the amounts indicated under Fig. 2. Phosphoserine was omitted from the control. At the times indicated, 1.0-ml samples were mixed with 0.1 ml of 50% trichloroacetic acid. Other conditions were as in Fig. 2.*

termining the amount of inorganic phosphate formed during the incubation period. With the extracts prepared from *E. coli* and *S. typhimurium*, magnesium appeared to be the divalent cation of choice. Figures 4 and 5 show that, with the assay system employed, the extent of the reaction was proportional to time of incubation and to extract concentration, respectively.

Activities of the enzymes of the 3-phosphoglycerate pathway in wild-type and serine-requiring strains of E. coli and S. typhimurium. To ascertain that the three enzymes in the 3-phosphoglycerate pathway are, in fact, obligatorily required for serine biosynthesis, it would be desirable to demonstrate that the loss of the capacity to form any one of the three enzymes results in the loss of the ability to form serine. Conversely, reversion to prototrophy would be invariably accompanied by the acquisition of the capacity to form the enzyme previously missing. Accordingly, these activities were measured in extracts prepared from wild-type and representative serine-glycine auxotrophic strains of *E. coli* and *S. typhimurium*.

As shown in Table 3, the extract prepared from

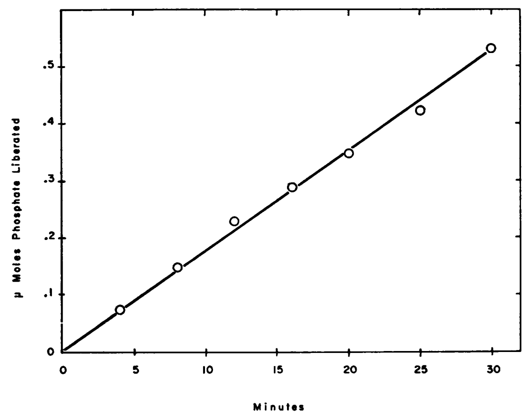

FIG. 4. *Liberation of inorganic phosphate from phosphoserine by an extract of Salmonella typhimurium strain ser B 4. The experimental tube contained, in 3.0 ml: 300 μmoles of tris-HCl buffer (pH 7.5), 90 μmoles of magnesium chloride, 120 μmoles of* DL-*phosphoserine, and bacterial extract containing 2.5 mg of protein. The tubes were incubated for 2 min before substrate was added. Samples (0.25 ml) were removed for the determination of inorganic phosphate. Temp: 37 C.*

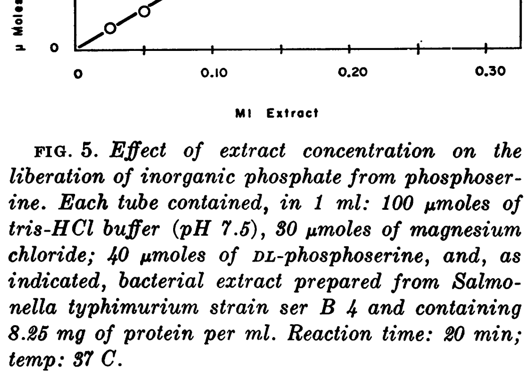

FIG. 5. *Effect of extract concentration on the liberation of inorganic phosphate from phosphoserine. Each tube contained, in 1 ml: 100 μmoles of tris-HCl buffer (pH 7.5), 30 μmoles of magnesium chloride; 40 μmoles of* DL-*phosphoserine, and, as indicated, bacterial extract prepared from Salmonella typhimurium strain ser B 4 and containing 8.25 mg of protein per ml. Reaction time: 20 min; temp: 37 C.*

TABLE 3. *Enzyme activities in wild-type and serine-glycine auxotrophic organisms*

Organism	Strain	Enzymatic activity of extract (μmoles per mg of protein per hr)		
		3-Phosphoglycerate dehydrogenase*	Glutamate phosphoserine† transaminase	Phosphoserine phosphatase‡
Escherichia coli	K-12	1.17	2.89	2.29
	119 HM 1	0.00	0.60	2.85
	129 HM 1	0.00	0.44	2.11
Salmonella typhimurium	LT-2	0.74	0.94	3.67
	ser A 4	0.00	1.35	3.35
	ser B 10	0.90	1.74	0.01

* For conditions, see Fig. 1.
† For conditions, see Fig. 2 and 3.
‡ For conditions, see Fig. 4 and 5.

the LT-2 strain of *S. typhimurium* and that prepared from the K-12 strain of *E. coli* each exhibited all three activities. Of several strains of *E. coli* serine-glycine auxotrophs examined, all exhibited both transaminase and phosphatase activities. Of these, the results of the examination of only the extracts prepared from strains 119 HM 1 and 129 HM 1 are shown in the table. In none of the serine-glycine auxotrophs of *E. coli* could 3-phosphoglycerate dehydrogenase activity be detected.

With the serine-glycine auxotrophs of *S. typhimurium*, biochemical differences might have been expected since representatives of two genetically distinct classes were chosen. This genetic distinction was discovered by Demerec and coworkers (1955), who examined a large number of serine-glycine auxotrophs derived from *S. typhimurium* by spontaneous and 2-aminopurine-induced mutations and found them all to exhibit lesions in one or the other of two unlinked cistrons, *ser A* and *ser B*. The biochemical examination of some of these strains has uniformly paralleled the genetic findings. The results obtained with extracts prepared from two typical representatives of these two groups, strains *ser A* 4 and *ser B* 10, are given in Table 3. It may be seen that indeed the two groups could be differentiated biochemically. While both strains exhibited transaminase activity, strain *ser A* 4 was blocked in the dehydrogenase and *ser B* 10 was blocked in the phosphatase. In all other strains examined, this same difference between *ser A* and *ser B* mutants has been found.

Discussion

From these data, it is quite clear that in *S. typhimurium* the pathway to serine from glucose includes 3-phosphoglycerate dehydrogenase and phosphoserine phosphatase as obligatory enzymes. Although it seems incontrovertible that a transamination is required for the conversion of phosphohydroxypyruvate to phosphoserine, mutant methodology does not yet permit a decision regarding the existence of a single enzymatic mechanism for this reaction. Thus, it is not yet possible to ascertain whether the phosphoserine-glutamate transamination observed in the extracts examined has been catalyzed by a single specific enzyme or by one or more enzymes with a rather broad spectrum of specificity.

A further observation which supports the obligatory nature of this pathway is that, following transduction by phage PLT22 grown on a *ser A* mutant, prototrophs derived from a *ser B* mutant could be shown to form phosphoserine phosphatase. Conversely, the capacity to form 3-phosphoglycerate dehydrogenase accompanied transduction to prototrophy of a *ser A* mutant by phage grown on a *ser B* mutant.

Strictly speaking, in *E. coli* it has been possible only to designate 3-phosphoglycerate dehydrogenase as an obligatory enzyme in the pathway leading to serine. However, because of the very great number of similarities in *S. typhimurium* and *E. coli*, it would be most surprising if there were significant species differences in this respect.

Further evidence that supports a similar pathway for the two species can be obtained by comparing the genetic map of *E. coli* K-12 with the results of the genetic experiments of Glanville and Demerec (1960). It was noted that the *ser B* locus was sufficiently close to four of five loci for threonine biosynthesis on the *S. typhimurium* chromosome that serine and threonine markers were jointly transduced with phage PLT22. The *ser A* locus, in contrast, was unlinked to any known markers. In *E. coli*, only one serine locus has been identified, and it has been placed on the chromosome map a considerable distance from a commonly employed threonine marker (Jacob and Wollman, 1961). Apparently, then, this locus, in which lesions appear to be common in *E. coli*, corresponds to the *ser A* locus in *S. typhimurium*. It is of interest, therefore, that the *E. coli* serine-glycine auxotrophs that have been examined are missing the same enzyme (3-phosphoglycerate dehydrogenase) that is missing in the *ser A* mutants of *S. typhimurium*.

For some time it has been known that *E. coli* contained a phosphoserine-glutamate transaminase and a phosphoserine phosphatase (Smith et al., 1956). However, from a review of the literature, it does not appear that any mutants requiring serine or glycine had been examined prior to the study reported. As emphasized previously, one of the few ways, and certainly the most direct, of establishing the obligatory nature of a biosynthetic pathway is by the finding that loss of the enzyme in question leads to auxotrophy for the end product of the pathway of which the enzyme is a part.

An additional feature of biosynthetic pathways is that they are commonly controlled by end product inhibition of the initial enzyme in the sequence and by repression of all the enzymes in the pathway. Although the serine-glycine auxotrophs employed in this study have usually been grown under conditions of limiting serine (or glycine), no definitive evidence has been obtained that the enzymes in the serine pathway are repressible and derepressible in the commonly observed fashion. This problem, however, is still being examined.

Regarding the question of control of metabolite flow by end product inhibition, preliminary, though definitive, evidence has already been obtained (Umbarger and Umbarger, 1962). Thus, in accord with the pattern so widely found, L-serine is an inhibitor of the first enzyme in the pathway, 3-phosphoglycerate dehydrogenase. It has been suggested from studies on phosphoserine phosphatase in animal tissues (Neuhaus and Byrne, 1958; Borkenhagen and Kennedy, 1958) that L-serine might control its biosynthetic pathway by the sensitivity of the phosphatase to serine. However, in the bacterial system studied here, appreciable inhibition of this enzyme is observed only when the concentration of serine approaches that required for saturation by substrate. In contrast, the first enzyme is about 1,000 times as sensitive to serine. A more thorough analysis of this interaction between the first enzyme and compounds inhibitory to it is in progress.

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